Rheedea Vol. 12(2): 89-99. 2002

A molecular systematic study of *Aralia* L. and *Panax* L. (Araliaceae) in India, and its taxonomic implications

Arun K. Pandey, Chunghee Lee* and Jun Wen*
University Department of Botany, TM Bhagalpur University, Bhagalpur - 812 007, Bihar, India
*Department of Botany, The Field Museum, 1400 S. Lake Shore Dr., Chicago 60605, Illinois, U.S.A.

Abstract

The internal transcribed spacer (ITS) regions of nuclear ribosomal DNA were sequenced from 33 accessions belonging to four genera (*Aralia, Brassaiopsis, Merrilliopanax* and *Panax*) to assess phylogenetic relationships of taxa distributed in different geographic regions. The ITS sequence data support the monophyly of both *Aralia* and *Panax* and show a close relationship between the two genera. Within *Aralia*, taxa of *Aralia* sect. *Dimorphanthus* (*Aralia armata, A. finlaysoniana, A. foliolosa, A. hiepiana* and *A. spinifolia*) form a strongly supported monophyletic group whereas the monophyly of *Aralia* and sect. *Pentapanax*, (*A. gigantea, A. leschenaultii* and *A. parasitica*) is only weakly suggested. *Panax assamicus* from India forms a clade with *P. bipinnatifidus, P. ginseng, P. shangianus, P. wangianus* and *P. variabilis* from the Sino-Himalyan region, as well as *P. quinquefolius* from North America. Molecular differentiation is detected among *Panax assamicus* from India, *P. variabilis* from Southwestern China, and *P. wangianus* from West-Central China, although these taxa have differentiated very little at the morphological level.

INTRODUCTION

Araliaceae (the ginseng family) includes about 50 genera and approximately 1500 species (Wen *et al.*, 2001a). The family is distributed mostly in tropics and subtropics (especially in southeastern and southern Asia and the Pacific islands), with some genera occurring in the temperate zone (e.g., *Aralia* L., *Hedera* L., *Oplopanax* (Torr. & Gray) Miq. and *Panax* L.). The family includes a number of medicinally important taxa, such as *Panax* (ginseng) and *Eletheurococcus* (Siberian ginseng). In India the family Araliaceae are represented by 15 genera distributed mostly in north and northeastern region. They are: *Aralia* (11 spp.), *Brassaiopsis* Decne. & Planch. (9 spp.) *Dendropanax* Decne. & Planch. (1 sp.),

Eleutherococcus Maxim. (3 spp.), *Gamblea* C.B. Clarke (1 sp.), *Hedera* L. (1 sp.), *Heteropanax* Seem. (1 sp.), *Macropanax* Miq. (1 sp.), *Merrilliopanax* Li (4 spp.), *Panax* (4 spp.), *Polyscias* J.R. & G.Forst. (2 spp.), *Schefflera* J.R. & G.Forst. (ca. 25 species), *Tetrapanax* K. Koch. (1 sp.), *Trevesia* Vis. (1 sp.), and *Tupidanthus* Hook. f. & Thoms. (1 sp.).

Aralia in India includes A. *armata* (Wall. ex Don) Seem., *A. cachemirica* Decne., *A. foliolosa* Wall. ex C.B. Clarke, *A. gigantea* J.Wen, A. *kingdon-wardii* J. Wen, *A. leschenaultii* (DC) J. Wen, A. *malabarica* Beddome, *A. parasitica* (D. Don) J. Wen, *A .subcordata* (Don) J. Wen, A. *thomsonii* Seem., and *A. tibetana* G. Hoo (see revision by Wen *et al.*, 2002). The genus *Pentapanax* Seem. has been merged with *Aralia* based on the characters intermediate between the two genera. This taxonomic treatment has been subsequently supported by morphological and molecular phylogenetic evidence (Wen, 2001a; Wen *et al.*, 2002).

Panax consists of approximately 18 species, of which about 16 are from eastern Asia and two from eastern north America (Wen & Zimmer, 1996; Wen, 2001 b; Yoo *et al.*, 2001). Among the Asiatic species, several Himalayan taxa have been problematic due to sympatry of morphologically distinct taxa and the existence of occasional morphological intermediates (Wen & Zimmer, 1996). *Panax pseudoginseng* Wall. was described by Wallich in 1829 based on specimens collected from central Nepal, and its circumscription has presented problems to many taxonomists. Hara (1970) recognized four taxa from Nepal based on rhizome, root, and leaf characters: *P. pseudoginseng* subsp. *pseudoginseng* and *P. pseudoginseng* subsp. *himalaicus* Hara var. *himalaicus, bipinnatifidus* (Seem.) H.L. Li and *angustifolius* (Burkill) H.L.Li. He broadly defined *P. pseudoginseng* as a widespread species in the Himalayas, China and Japan. Hoo and Tseng (1973, 1978) followed Hara's species concept and made a few nomenclatural changes at the varietal level within *P. pseudoginseng*. Zhou *et al.* (1975), however, defined *P. pseudoginseng* narrowly, sensu Wallich (1829).

Wen and Zimmer (1996) reported that *P. pseudoginseng* of central Nepal is highly distinct with respect to both the profile of internal transcribed spacer (ITS) sequences and morphology. Watanabe *et al.* (1998) examined the morphology, RAPD profile, and saponin contents of *P. pseudoginseng* and its allied groups from Nepal and Japan. Their phylogenetic tree shows that the Himalyan *Panax* is distinct from the Japanese populations. Wen and Nowicke (1999) found that the pollen ultrastructure of *P. pseudoginseng sensu* Wall. (= *P. pseudoginseng* subsp. *pseudoginseng* of Hara) is different from that of Hara's *P. pseudoginseng* subsp. *japonicus* from Japan (=*P. japonicus* C.A. Meyer and from China (= *P. major* Ting and *P. sinensis* J. Wen. Choi and Wen (2000) reconstructed a phylogeny of *Panax* using cpDNA restriction site and nuclear rDNA ITS sequence data. This study also shows a distinct cpDNA profile of *P. pseudoginseng* sensu Wallich (1829), in comparison with that of the other taxa in the genus, thus supporting a narrowly defined *P. pseudoginseng* sensu Wallich. The taxonomy of Indian *Panax* is highly controversial (cf. treatments by Banerjee, 1968; Wen, 2001a) and the relationship of Indian Araliaceae taxa are not well understood.

Hence this study was undertaken to compare sequences of the internal transcribed spacer regions of nuclear ribosomal DNA among taxa, especially *Aralia* and *Panax* from India, China, and Nepal to detect patterns of evolutionary differentiation among taxa from different

geographic regions. This is a pilot molecular systematic study for plants in India using DNA markers and phylogenetic methods.

MATERIALS AND METHODS

Sequences of 32 accessions of *Aralia* and *Panax* were analyzed in this study (Table 1). *Brassaiopsis mitis* C.B. Clarke, *Merrilliopanax alpinus* (C.B. Clarke) C.B. Shang and *Osmoxylon geelvinkianum* Becc. were used as the outgroups. Voucher specimens are deposited at the Arnold Arboretum (A), Bhagalpur University herbarium (BHAG), Colorado State University herbarium (CS), Field Museum (F), and Missouri Botanical Garden (MO).

Table 1. Plant accessions used for the Indian Araliaceae study

Species	Voucher	Locality	GenBank Accession Number
1	2	3	4
Aralia armata (Wall. ex Don) Seem.	*Wen 6068* (F)	Lao Cai, Vietnam	AY233310
A. finlaysoniana (Wall. ex Don) Seem.	*Wen 1205* (F)	Yunnan, China	AY233311
A. foliolosa Seem.	*Pandey 5009D* (BHAG)	West Bengal, India	AY233312
A. lihengiana J. Wen *et al.*	*Wen 5707* (F)	Yunnan, China	AY233315
A. gigantea .J. Wen	*Pandey 5001D* (BHAG)	West Bengal, India	AY233313
A. gigantea J. Wen	*Pandey 5002D* (BHAG)	West Bengal, India	AY233314
A. hiepiana J. Wen & Lowry	*Lowry 4925* (F)	Lam Dong, Vietnam	AY233316
A. leschenaultii (DC.) J. Wen	*Pandey 5003B* (BHAG)	West Bengal, India	AY233318
A. leschenaultii (DC.) J. Wen	*Wen 5820* (F)	Yunnan, China	AY233317
A. parasitica (D. Don) J. Wen	*Wen 5744* (F)	Yunnan, China	AY233319
A. spinifolia Merr.	*Wen 1247* (F)	Guangdong, China	U41676

1	2	3	4
Brassaiopsis mitis C.B. Clarke	*Pandey 5005E* (BHAG)	West Bengal, India	AF551726
Merrilliopanax alpinus (C.B. Clarke) C.B. Shang	*Pandey 5008D* (BHAG)	West Bengal, India	AY233309
Osmoxylon geelvinkianum Becc.	*Plunkett 1489* (MO)	New Guinea	AF229727
Panax assamicus Banerjee	*Pandey 5017* (BHAG)	Meghalaya, India	AY233321
P. assamicus Banerjee	*Pandey 5018* (BHAG)	Meghalaya, India	AY233322
P. assamicus Banerjee	*Pandey 5000H* (BHAG)	West Bengal, India	AY233320
P. bipinnatifidus Seem.	*Wen 4942-5* (F)	Sheopore, Nepal	AY233323
P. bipinnatifidus Seem.	*Wen 5049* (F)	Yunnan, China	AY233324
P. bipinnatifidus Seem.	*Wen 5702-8* (F)	Yunnan, China	AY233325
P. ginseng C.A. Meyer	*Wen 3127* (F)	Jilin, China	AY233326
P. notoginseng F.H. Chen *ex* C.Y. Wu & K.M. Feng	*Wen 1244* (F)	Guangdong, China	U41685
P. pseudoginseng Wall.	*Wen 4900* (F)	Jiri, Nepal	AY233327
P. quinquefolius L.	*Wen 1083* (A)	Ohio, USA	U41687
P. shangianus J. Wen	*Wen 5075-8* (F)	Yunnan, China	AY233328
P. stipuleanatus H.T. Tsai & K.M. Feng	*Wen 1204* (F)	Yunnan, China	U41696
P. trifolius L.	*Kramer & Kramer s.n. (CS)*	Ohio, USA	U41698
P. wangianus S.C. Sun	*Wen 1174* (CS)	Sichuan, China	U41690
P. wangianus S.C. Sun	*Wen 1176* (CS)	Sichuan, China	U41691
P. variabilis J. Wen	*Wen 5693-4* (F)	Yunnan, China	AY233331
P. variabilis	*Wen 5694-11* (F)	Yunnan, China	AY233330
P. variabilis	*Wen 5695-1* (F)	Yunnan, China	AY233329

Total DNA was extracted with the CTAB method of Doyle and Doyle (1987) and purified over CsCl/ethidium bromide gradients. DNA amplifications were performed in 100μl reactions containing approximately 50ng genomic DNA, 20nM Tris buffer (pH 8.3) with 50mM KCl, 1.5mM $MgCl_2$, and 0.1% Tween 20 (buffer designed by C. Bult), 0.15mM of each dNTP, 1μM of each primer, 5 units of Taq DNA polymerase (Promega Co., Madison, Wisconsin, USA), and 5% DMSO (dimethyl sulfoxide). The ITS regions were amplified following Wen and Zimmer (1996), using different primers (Table 2). Double-stranded PCR products were produced via 45 cycles of denaturation (94°C for 1 min.), annealing (50°C for 2 min.), and extension (72°C for 2 min.). A 5-min final extension cycle at 72°C followed the 45 cycle to ensure the completion of novel strands. The PCR products were purified using Wizard PCR Preps DNA Purification System (Promega Co., Madison, Wisconsin, USA) prior to sequencing.

Table 2. Primers used for PCR amplification and sequencing reactions in present study
("f" and "r" added to published names to denote use as forward or reverse primers)

Locus	Primer	Sequence
ITS	ITSI (f)	5'-GTCCACTGAACCTTATCATTTAG-3'
	ITS4 (r)	5'-TCCTCCGCTTATTGATATGC-3'
	ITS5 (f)	5'-GGAAGTAAAAGTCGTAACAAGG-3'
	C5.8S (r)	5'-TGCGTTCAAAGACTCGAT-3'
	N5.8S (f)	5'-ATCGAGTCTTTGAACGCA-3'

The sequencing reaction was performed in a 10μl final volume using the BigDye Terminator cycle sequencing kit (Perkin-Elmer, Applied Biosystems) following the manufacturer's instructions. Sequenced product was precipitated with 10μl of deionized sterile water, 2μl of 3M NaOAC, and 50μl of 95% EtOH. Polyacrylamide gel electrophoresis was conducted using Long Ranger Singel packs (FMC BioProducts) and an ABI 3100 automated DNA sequencer (Perkin-Elmer, Applied Biosystems). The resulting sequence were assembled using Sequencher® (ver. 3.1.1) and aligned manually.

Phylogenetic analyses were performed with PAUP* using the maximum parsimony (Swofford *et al.*, 1996). Parsimony analysis was performed using a branch-and-bound search with MULPARS and furthest addition sequence options. The amount of support for monophyletic groups revealed in the maximally parsimonious tree(s) (MPTs) was examined with 100 bootstrap replicates (Felsenstein, 1985) with the random addition and the heuristic search options using parsimony.

RESULTS

Characteristics of ITS sequences

The combined length of the entire ITS region (ITS1, 5.8S and ITS2) from taxa sampled in the present study ranged from 657 to 687bp. The ITS1 region was 257 bp in length, the 5.8S gene was 163 bp and the ITS2 region was 267 bp. Insertions or deletions (indels) were necessary to align the 26 sequences. Of these indels 10 were located in the ITS1 region, and 10 in ITS2 region. The indels ranged in length from 3 to 15 bp.

Phylogenetic Analyses

The parsimony analysis of the entire ITS region resulted in 378 maximally parsimonious trees (MPTs) with a total length of 266 steps, a consistency index (CI) of 0.759 (0.627 excluding uninformative characters) and a retention index (RI) of 0.858. The strict consensus tree, and one of the MPTs are presented in Figs. 1 and 2, respectively. The bootstrap values were indicated in Fig. 1 to show the relative support of each clade.

DISCUSSION

All trees resulting from the analysis of ITS sequences resolve two major clades, representing *Aralia* and *Panax*, respectively (Figs. 1,2).

Aralia consists of six taxonomic sections (Wen, 1993). Two of the sections (sect. *Dimorphanthus* and sect. *Pentapanax*) occur in India and are included in the present analysis. In India, *Aralia* Sect. *Dimorphanthus* is represented by four species. (A. *armata,* A. *foliolosa,* A. *malabarica* and *A. thomsonii).* Of these, A. *malabarica* is endemic to South India whereas the remaining three species are widely distributed in Asia (Wen *et al.*, 2002).

In India, *Aralia* sect. *Pentapanax* is represented by five species (A. *gigantea, A. kingdon-wardii, A. leschenaultii, A. parasitica* and *A. subcordata).* Three species of sect. *Pentapanax (A. gigantea, A. leschenaultii,* and *A. parasitica)* are included in the present study. These three taxa are weakly supported to be monophyletic in only some of the 378 most parsimonious trees. Apparently the three Indian species of the section are evolutionarily distantly related to each other. Morphologically *Aralia subcordata* (not sampled in the study) is closely related to *A. gigantea,* both sharing the synapomorphies of a racemose inflorescence unit, and small floral parts and fruits.

Aralia sect. *Dimorphanthus* consists of 29 species, of which two occur in eastern North America (*A. hispida* Vent. and *A. spinosa* L.) and remaining species in Asia extending from eastern Russia to northern New Guinea (Wen, 2001a). *Dimorphanthus* Miq. was originally described (Miquel, 1840) as a monotypic genus (including *D. elastus)* from Japan, distinct from the Linnaean *Aralia.* Miquel (1856a) later reduced it to a rank of subgenus within *Aralia.* However, he soon relegated the group to sect. *Dimorphanthus* (Miquel, 1856b) and

A molecular systematic study of *Aralia* L. and *Panax* L.

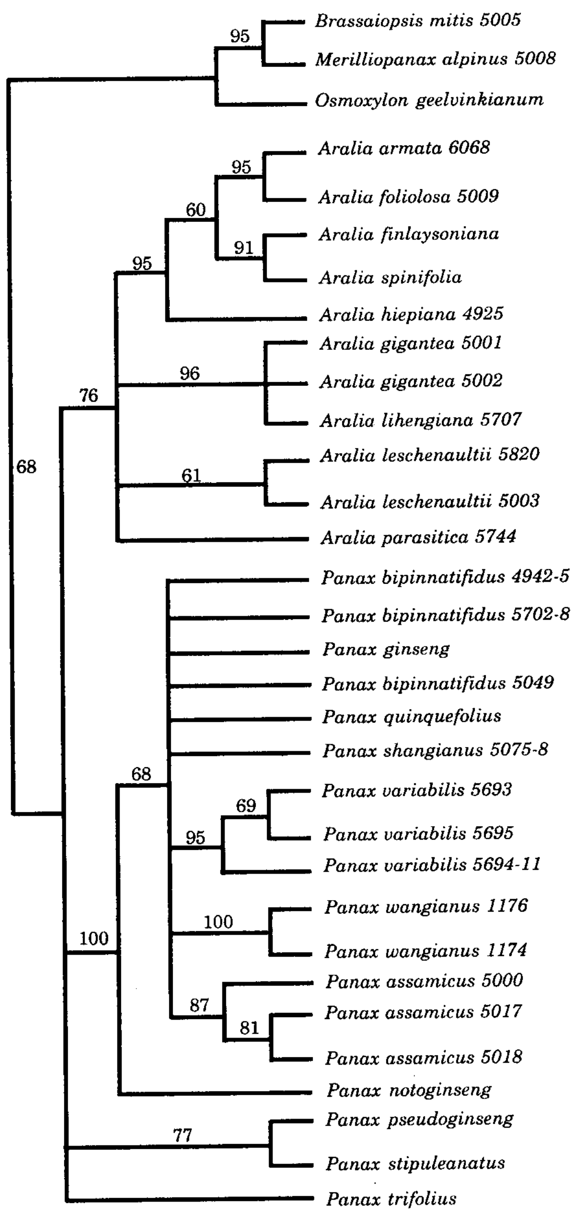

Fig. 1. The strict consensus tree of the ITS phylogeny of *Aralia* and *Panax* from India. (Numbers above the branches are bootstrap values).

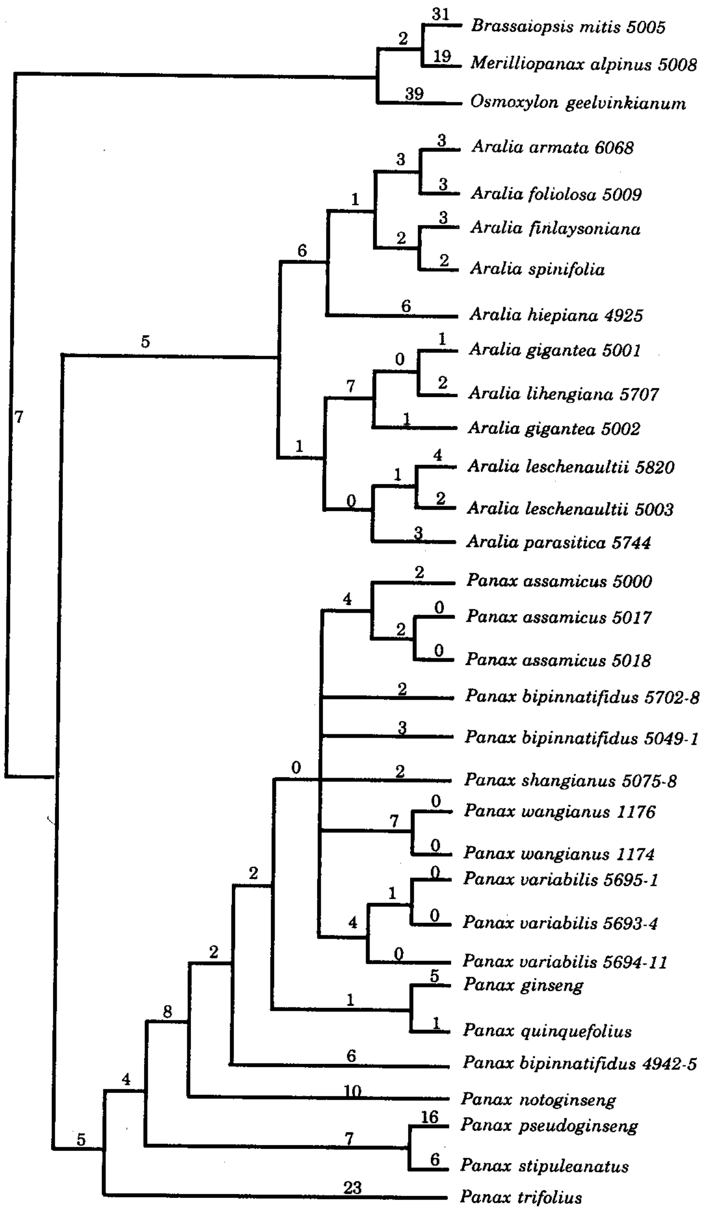

Fig. 2. One of the 378 maximally parsimonius trees of *Aralia* and *Panax* from India based on the ITS sequences of nuclear ribosomal DNA. (Numbers above the branches indicate branch lengths).

used this concept con)sistently in his later works (e.g. Miquel, 1857, 1863). The ITS phylogeny of *Aralia-Panax* complex conducted by Wen (2001a) shows that *Aralia* sect. *Dimorphanthus* is paraphyletic and within this section *Aralia* sect. *Pentapanax* is also nested. In the present study, however, taxa of both sect. *Dimorphanthus* and sect. *Pentapanax* form two different clades. This discrepancy may be due to the fact that two basally branching taxa of *Aralia* sect. *Dimorphanthus: A. spinosa.*and *A. hispida.* were not included in the present study.

Aralia sect. *Pentapanax* consists of approximately 15 species from subtropical, tropical and warm temperate regions of Asia. The section was previously treated at the generic rank as *Pentapanax* Seem. (Seemann, 1868). A close relationship between *Aralia* and *Pentapanax* has been hypothesized (Harms, 1898; Hoo, 1961; Hoo & Tseng, 1978). Wen (1993) merged the genus *Pentapanax* with *Aralia* and established a separate section *Pentapanax.* The ITS phylogeny of the entire genus *Aralia* (Wen, 2000, 2001a) shows that taxa earlier recognized as *Pentapanax* [e.g., *Pentapanax fragrans* (DC.) Ha and *P.racemosus* Seem.)] were nested within *Aralia*, a feature also observed in earlier broader studies of core Araliaceae (see Wen *et al.*, 2001). Specifically, taxa of sect. *Pentapanax* were embedded within *Aralia* sect. *Dimorphanthus.* Our present study with a smaller sampling scheme also suggests a close relationship between the two sections.

The taxonomy of *Panax* in the Himalayan region has been highly controversial. Hara (1970) treated all Himalayan *Panax* as one species: *P.pseudoginseng.* Recent molecular and morphological studies (e.g., Wen & Zimmer, 1966; Choi & Wen, 2000; Wen, 2001b; Wen *et al.*, 2001; Yoo *et al.*,2001) suggest that *P. pseudoginseng* should be narrowly defined to include populations of *Panax* in central Nepal and Tibet with tap roots and persistent stipules. Our study supports that *P. pseudoginseng s.str.* is distinct from other *Panax* populations with elongated rhizomes of the Himalayan region as well as from China (e.g., *Panax bipinnatifidus* and *P. assamicus;* cf. Figs. 1-2).

Panax assamicus was described by Banerjee (1968) on material from the Shillong area, India. Its species status has been questioned by Hara (1970) and Wen (2001b). Morphologically *P. assamicus* is very similar to *P.wangianus* Sun from West Central China, and to *P. zhengyianus* J. Wen from southwestern China. These three taxa all have narrow leaflets, elongated rhizomes with thick and short internodes, and fruits turning into black except a small area near the pedicel. The ITS data suggest that the Indian *P. assamicus* is clearly distinct from the morphologically similar *P. wangianus* from West-Central China, and *P. zhengyianus* from southwestern China. We thus herein recognize *Panax assamicus* as a distinct species.

Acknowledgements

This study was supported by the Bass Fellowship of The Field Museum to A. K. Pandey, the postoctoral fellowship from the Korean Science and Engineering Foundation to

Chunghee Lee, and the U.S. National Science Foundation (DEB 0108536 and DEB 0196051) to Jun Wen.

Literature Cited

Banerjee, R. N. 1968. A taxonomic revision of Indian *Panax* L. *Bull. Bot. Survey India* 10 (1): 20-27.

Choi, H. K. & J. Wen. 2000. A phylogentic analysis of *Panax* (Araliaceae): integrating evidence of chloroplast DNA and the ITS sequences of nr DNA. *Pl. Syst. Evol.* 224: 109-120.

Doyle, J. J. & J. I. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bull.* 19: 11-15.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the boot strap. *Evolution* 39: 783-791.

Hara, H. 1970. On the Asiatic species of the genus *Panax*. *J. Jap. Bot.* 45: 197-212.

Harms, H. 1898. Zur kenntnis der Gattungen *Aralia* und *Panax*. *Bot. Jahrb. Syst.* 23: 1-23.

Hoo, G. 1961. The systematics, relationship and distribution of the Araliaceae of China. *Bull. Xiamen Univ. (Nat. Sci.)* 8(1): 1-11.

Hoo, G. & C. J. Tseng. 1973. On the Chinese species of *Panax* Linn. *Acta Phytotax. Sin.* 11: 431-438.

Hoo, G & C.J. Tseng. 1978. *Angiospermae, dicotyledoneae, Araliaceae. Flora Reipublicae Popularis Sinicae.* Vol. 54. Science Press, Beijing. [In Chinese].

Miquel. F. A. W. 1840. *Commentarii Phytographici.* Leiden.

Miquel, F. A. W. 1856a [1 May 1856]. Araliacearum indicarum genera et species aliquod novae. *Bonplandia* 4: 137-139.

Miquel, F. A. W. 1856b [10 July 1856]. Araliaceae. *Fl. Ned. Ind.* 1:745-769.

Miquel, F. A. W. 1857. Araliaceae. *In:* W.H. de Vries (Ed.), *Plantae Indiae batavae orientalis.* Vol. 2. E. J. Brill, Lugduni-Batavorum. pp. 81-92.

Miquel, F. A. G. 1863. *Oplopanax. Ann. Mus. Bot. Lugduni-Batavorum* 1:16.

Seemann, B. 1868. *Revision of the natural order of Hederaceae.* L. Reeve & Co., London.

Swofford, D. L., G. J. Olsen, P. J. Waddell & D. M. Hillis. 1996. *Phylogenetic inference. In:* D.M. Hillis, C. Moritz and B. K. Mable (Eds.), *Molecular Systematics.* pp. 407-514.

Wallich, N. 1829. An account of the Nipal ginseng. *Trans. Med. Phys. Soc. Calcutta* 4: 115-120.

Watanabe, T., K. Kawaguchi, H. Suzuki, T. Yoshikawa, A. Takano, S. Isoda, H. Kohda & K.J. Malla. 1998. Studies on the medicinal plant resources of the Himalayas 93), random amplified

polymorphic DNA analysis and saponin contents of Himalayan ginseng (*Panaxpseudo-ginseng* Wall.). *Nat. Med.*52:426-433.

Wen, J. 1993. Generic delimitation of *Aralia* (Araliaceae). *Brittonia* 45: 47-55.

Wen, J. 2000. Internal transcribed spacer phylogeny of the Asian-Eastern North American disjunct *Aralia* sect. *Dimorphanthus* (Araliaceae) and its biogeographic implications. *Intl. J. Pl. Sci.* 161: 959-966.

Wen, J. 2001a. Evolution of the *Aralia-Panax* complex (Araliaceae) as inferred from nuclear ribosomal ITS sequences. *Ebinborough J. Bot.* 58: 243-257.

Wen, J. 2001b. Species diversity, nomenclature, phylogeny, biogeography and classification of the ginseng genus (*Panax* L., Araliaceae). *In:* Z.K. Punja (Ed.), *Proc.International Ginseng Workshop.* Simon Fraser University, Burnaby, Canada. pp.67-88.

Wen, J. & E.A. Zimmer. 1996. Phylogeny and biogeography of *Panax* L. (the ginseng genus, Araliaceae): inferences from ITS sequences of nuclear ribosomal DNA. *Molec. Phylog. Evol.* 6: 166-177.

Wen, J. & J. W. Nowicke. 1999. Pollen ultrustructure of *Panax* L. (the ginseng genus, Araliaceae), an eastern Asian and eastern North American disjunct genus. *Amer. J. Bot.* 86: 1624-1636.

Wen, J., A. K. Pandey & M. K. Pathak. 2002. Revision of *Aralia* L. (Araliaceae) in India. *Rheedea* 12(1): 1-20.

Wen, J., G. M. Plunkett, A. Mitchell & S. Wagstaff. 2001. The evolution of Araliaceae: a phylogenetic analysis based on ITS sequences of nrDNA. Syst. Bot. 26 (1): 144-167.

Yoo, KI-OUG, K. J. Malla & J. Wen. 2001. Chloroplast DNA variation of *Panax* (Araliaceae) in Nepal and its taxonomic implications. *Brittonia* 53 (3): 447-453.

Zhou, J., W. G. Huang, M. Z. Wu., C. R. Yang, K. M. Feng, & Z. Y. Wu. 1975. Triterpenoids from *Panax* L. and their relationship with taxonomy and geographical distribution. *Acta Phytotax. Sin.* 13(2): 29-45.

polymorphic DNA analysis and saponin contents of Himalayan ginseng (*Panaxpseudo-ginseng* Wall.). *Nat. Med.*52:426-433.

Wen, J. 1993. Generic delimitation of *Aralia* (Araliaceae). *Brittonia* 45: 47-55.

Wen, J. 2000. Internal transcribed spacer phylogeny of the Asian-Eastern North American disjunct *Aralia* sect. *Dimorphanthus* (Araliaceae) and its biogeographic implications. *Intl. J. Pl. Sci.* 161: 959-966.

Wen, J. 2001a. Evolution of the *Aralia-Panax* complex (Araliaceae) as inferred from nuclear ribosomal ITS sequences. *Ebinborough J. Bot.* 58: 243-257.

Wen, J. 2001b. Species diversity, nomenclature, phylogeny, biogeography and classification of the ginseng genus (*Panax* L., Araliaceae). *In:* Z.K. Punja (Ed.), *Proc.International Ginseng Workshop.* Simon Fraser University, Burnaby, Canada. pp.67-88.

Wen, J. & E.A. Zimmer. 1996. Phylogeny and biogeography of *Panax* L. (the ginseng genus, Araliaceae): inferences from ITS sequences of nuclear ribosomal DNA. *Molec. Phylog. Evol.* 6: 166-177.

Wen, J. & J. W. Nowicke. 1999. Pollen ultrustructure of *Panax* L. (the ginseng genus, Araliaceae), an eastern Asian and eastern North American disjunct genus. *Amer. J. Bot.* 86: 1624-1636.

Wen, J., A. K. Pandey & M. K. Pathak. 2002. Revision of *Aralia* L. (Araliaceae) in India. *Rheedea* 12(1): 1-20.

Wen, J., G. M. Plunkett, A. Mitchell & S. Wagstaff. 2001. The evolution of Araliaceae: a phylogenetic analysis based on ITS sequences of nrDNA. Syst. Bot. 26 (1): 144-167.

Yoo, KI-OUG, K. J. Malla & J. Wen. 2001. Chloroplast DNA variation of *Panax* (Araliaceae) in Nepal and its taxonomic implications. *Brittonia* 53 (3): 447-453.

Zhou, J., W. G. Huang, M. Z. Wu., C. R. Yang, K. M. Feng, & Z. Y. Wu. 1975. Triterpenoids from *Panax* L. and their relationship with taxonomy and geographical distribution. *Acta Phytotax. Sin.* 13(2): 29-45.